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Cytogenetics of *Lolium perenne*

Part 3. Correlation between chiasmata and U-type exchange

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Summary. In two contrasting types of S5 inbred *Lolium perenne* a correlation has been found between the distribution pattern of chiasmata within bivalents and that of meiotic U-type exchange. The relationship between the two processes is discussed, in terms of inbreeding effects upon chiasma redistribution, and mention is also made of some other types of chromosome breakages which arise following enforced self-pollination.

Key words: *Lolium perenne* – Meiosis – Chiasma distribution – U-type exchange

Introduction

Lolium perenne is a strictly outbreeding species with a high level of heterozygosity and a well adapted meiotic mechanism which regulates the release of its variation. In terms of chromosome behaviour at meiosis the chiasmata are formed mainly in the distal segments of the bivalents and their distribution among chromosome pairs within pollen mother cells (pmcs), as well as between different pmcs within a plant, is highly uniform and under strict genetic control. The rigour of this control is revealed when plants are forcibly self-pollinated and inbred lines are developed which expose homozygous combinations of polygenes lacking in the normal balance and effectiveness. The inbreeding results in a progressive reduction in pmc chiasma frequency and an increase in the cell and bivalent variances (Karp and Jones 1982). There is also a loss of control over the distal localisation of chiasmata within bivalents, and they are found to occur more frequently in the interstitial and proximal locations (Karp and Jones 1983). In the first two publications in this series these cytological aspects of the recombination system were presented in detail and a two-level model of genetic control over the components of chiasma variation was proposed. In this paper we further analyse the aspect of chiasma distribution within bivalents, and consider its relationship to the phenomenon of U-type meiotic exchange which is also a characteristic feature (defect) of chromosome behaviour associated with, and revealed by, close inbreeding.

It was noticed when analysing the S5 generation inbred plants that a number of individuals had quite a high frequency (up to 19%) of bridge and fragment (B+F) configurations at anaphase I (AI). When any one of these individuals was examined in detail it was found that the fragments varied in length from cell to cell, and from this observation it was inferred that the B+Fs were the result of U-type exchange events. The term U-type exchange, as used here, refers to the process whereby breaks in non-sister chromatids in the paired homologues are followed by U-type reunion rather than the criss-cross reciprocal breakage and rejoining which occurs in normal chiasma formation. It results in the formation of a dicentric chromatid which gives a bridge at AI, and an acentric fragment (Fig. 2).

B+Fs can also occur as a result of inversion heterozygosity. For a given inversion, however, the fragment released at AI is always of the same size: this is because it is always generated in the same way and can be traced at pachytene (in theory at least) from the end of the chromosome arm – round the inversion loop – through the chiasma and back to the end of the

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chromosome arm. Since, for a given inversion, the loop will always be in the same place the fragment is always of the same length and, consequently, it becomes necessary to postulate a large number of different inversions in order to account for variability in fragment size. This seems most unlikely in the present material as there is no evidence of any inversions within the original parent plants and the inbreeding itself would in any case tend to eliminate the structural heterozygotes.

U-type breakage and reunion can involve sister, as well as non-sister, chromatids and these sister U-types may also lead to bridge and fragment formation (Rees and Thompson 1955; Jones 1968). In the present material however we can find no evidence for any significant level of sister U-type exchanges: characteristically they result in asymmetrical bivalents at metaphase I (MI) when a chiasma is formed distal to the sister breakage and reunion event, and we are familiar with their appearance in other genotypes and pedigrees within the inbred stocks. In addition the sister type of breakage and reunion give rise to AI chromatid loops (and fragments) which later form distinctive bridges at AII (Jones and Brumpton 1971). In the present material such AII bridges were conspicuously absent.

It seems reasonable therefore to accept that the B+Fs are the result of non-sister U-type exchanges, and that the variation in fragment sizes is due to differences in the location in which the exchanges occur. Furthermore, by measuring a sample of fragment lengths it is possible to determine the distribution of the breakages with respect to the proximal, interstitial and distal segment of the chromosomes. If, as is claimed in rye (Jones 1968, 1969), the U-type exchanges result from errors in chiasma formation then their distribution pattern should coincide with that of chiasmata. This hypothesis can easily be tested in *L. perenne* using S5 inbred lines from pedigrees which are known to differ in their chiasma distribution pattern. If the U-type exchanges are the result of errors in chiasma formation then chiasmata localised in the distal, interstitial and proximal regions of the bivalents would be expected to yield small, medium sized and large fragments respectively, following their defective expression as U-type exchange events. If, on the other hand, U-type exchange is independent of chiasma formation there should be no such correspondence. In addition of course, if the coincidence in distribution of the two kinds of events is confirmed, and the error theory accepted, the analysis will then provide some evidence (albeit indirect) that the change in chiasma position contingent upon inbreeding represents a real shift in the sites of their formation, rather than some artefact due to delayed terminalisation effects.

In this publication a small section is also included on some of the other types of chromosome breakage events which have arisen during the inbreeding programme.

Materials and methods

The material consists of several S5 generation plants of *L. perenne* var. S23 belonging to two of the four pedigrees of inbred lines (families) described in the two previous papers in this series (Karp and Jones 1982, 1983). In particular we have used four P15 S5 plants – two from each of two families, and four P30 S5 plants – two from one family and two from two others.

Analysis of meiotic chromosomes, as before, was made on pmcs from inflorescences fixed in Carnoy's fluid. The distribution of chiasmata within the bivalents was determined from samples of fifty MI cells in each plant, and they were classified into the three categories of distal, interstitial and proximal as already described (Karp and Jones 1983). Fragment sizes were measured using the same plants by sampling 50 AI cells with the bridge and fragment configuration. The fragments were grouped into three classes of equal size intervals and designated as small, medium or large. Percentage data were all transformed to angles for analysis. The additional material referred to in the section on other types of chromosome breakage comes from the same breeding programme but has not been previously described in the papers in this series: its ancestry is given in detail in Jones and Janabzadeh (1981).

Results

Correlations of chiasmata and U-type exchanges

The data on mean percentages of proximal, interstitial and distal chiasmata, from the 50 MI pmcs sampled in each plant, are given in Table 1 and in the histograms in Fig. 1. The distributions are very much as expected from the previous S5 analysis (Karp and Jones 1983). P15 has the greater proportion of chiasmata in the distal segments of the chromosome arms and correspondingly reduced numbers in the interstitial and proximal regions. P30 in contrast has far fewer distals (20% less than P15) and a much higher proportion in the interstitial and proximal regions. Table 1 also contains data on the frequency of occurrence of AI pmcs showing the bridge and fragment configurations, some examples of which are shown in Fig. 2. The incidence of B+Fs is a little higher in the P15 (15.7%) than in the P30 (13.9%) pedigree plants. In a few of the AI cells more than one B+F was present (Fig. 2c) in these cases both of the fragments were measured, although the cells were still recorded as having one B+F. The same holds for the rare cases of a double bridge and two fragments (Fig. 2d), where again both of the fragments were measured. Fragment sizes ranged from 0.3–2.2 μ in the P15 plants and from 0.3–2.7 μ in

Table 1. Frequency of *AI pmcs* with bridges and fragments $B+F^a$, together with percentages of proximal *prox.*, interstitial *int.* and distal *dist.* chiasmata classes, grouped respectively with the large *L* medium *M* and small *S* fragment classes in the four plants of the *P15* and *P30* pedigrees

S5 Plants	% pmcs with B+F	Percentages					
		Prox.	L	Int.	M	Dist.	S
P 15 a	15.63	4.35	5.66	14.87	24.53	80.78	69.80
b	14.41	1.57	1.92	13.91	13.46	84.52	84.62
c	18.12	1.78	9.80	13.35	17.65	84.87	72.55
d	14.79	3.40	1.92	12.34	26.92	84.26	71.15
Mean	15.74	2.78	4.81	13.62	19.71	83.61	75.50
P 30 a	19.08	12.75	7.40	26.40	33.33	60.85	61.10
b	6.57	8.05	8.70	33.47	32.60	58.47	58.70
c	16.79	8.52	11.76	40.81	35.29	50.67	52.94
d	13.19	5.62	9.80	23.60	25.49	70.78	64.71
Mean	13.91	8.74	9.55	31.07	30.65	60.19	59.80

[a] Pmcs with more than one B+F, or with a double bridge and two fragments were counted as one AI with B+F

P30. For each plant the fragments were classified into three groups of equal size intervals over the range 0.3–2.7 μ. Those less than 1.1 μ were designated as small (S), 1.1–1.9 μ as medium (M) and 1.9–2.7 μ as large (L); the data are also presented in Table 1. In the histograms in Fig. 1 the frequencies are given in 0.1 μ intervals in order to show the range and distribution of sizes, and in terms of S, M and L to show their coincidence with the three types of chiasma distribution.

In making this comparison of course it is important to establish that the two sets of data are comparable in terms of their relationship to absolute chromosome arm length, and that the largest fragments do in fact arise from breakages proximal to the centromere. Confirmation comes from comparing lengths of AI chromosomes with those of the large fragment group (L). The mean chromosome length averaged over five cells from each of a P15 and P30 plant, came out at 2.7 μ; the range of the L fragments was from 1.9–2.7 μ. The largest fragments found therefore arose from breakage adjacent to the centromere – bearing in mind that the chromosomes are mostly metacentric or submetacentric, and that the fragment length itself is twice the distance between the exchange point and the end of the arm when its two chromatids are opened out at AI.

Analysis of variance on the data in Table 1 gives highly significant differences between the three chiasma distribution classes (prox., int., dist.) and also between the fragment size groups (L, M, S); $P < 0.001$ in both cases. The interaction item classes/pedigrees is also significant for both ($P < 0.01$), confirming the difference in distribution pattern between the two pedigrees. Coincidence of the two patterns is confirmed by a high level of significance for the correlation coefficient for chiasma types and their corresponding fragment size

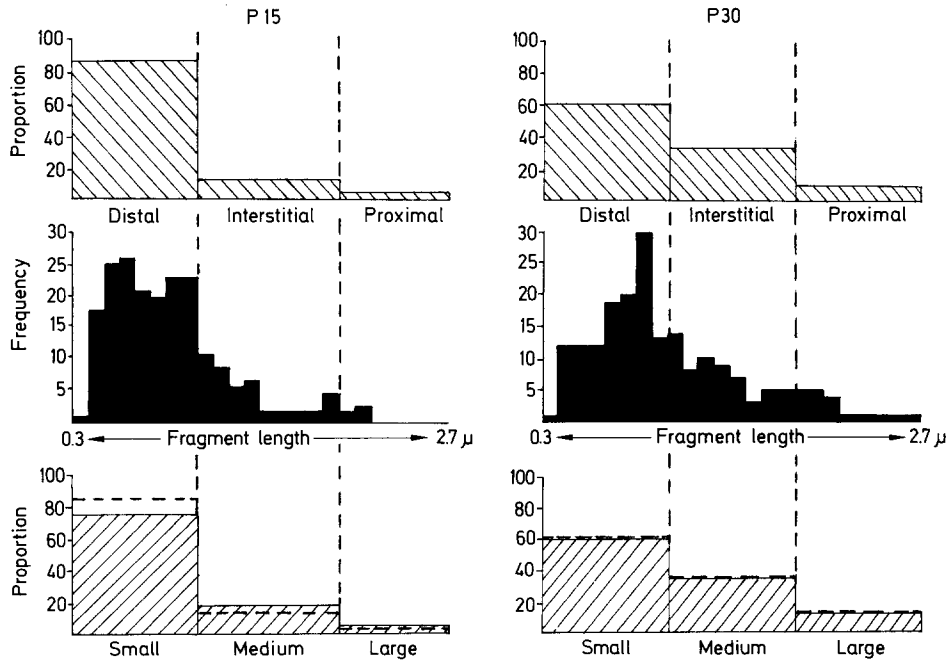

Fig. 1. Histograms showing the distribution of mean chiasma classes (*top row*), mean fragment sizes (*centre*) and the coincidence between them (*bottom row*), in the P15 and P30 S5 plants

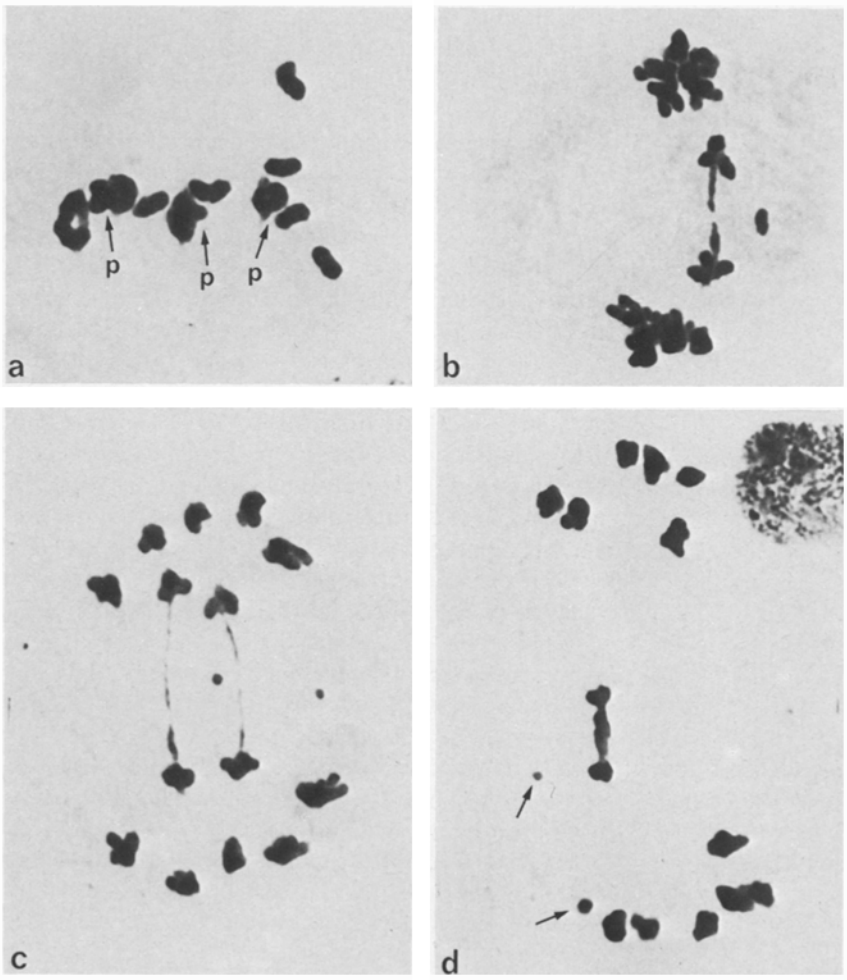

Fig. 2 a – d. Meiosis in P30 S5 showing **a** MI with a high proportion of non-distal chiasmata (*arrowed*); **b** AI with a bridge and large fragment; **c** AI with two bridges and two small fragments of different sizes; **d** AI with a double bridge and two fragments of different sizes (*arrowed*)

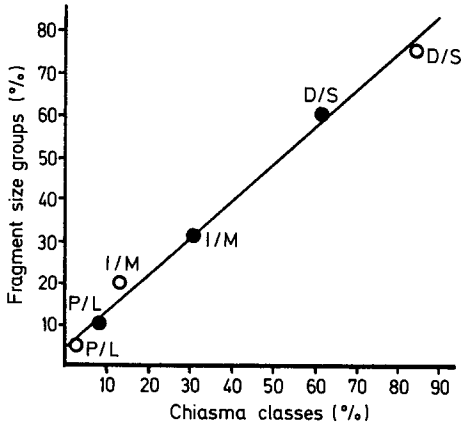

Fig. 3. Regression line, based on the combinations of means in Table 1, showing the close relationship between chiasma types (proximal *P*; interstitial *I*; distal *D*) and their corresponding fragment size groups (*L, M, S*) in the two pedigrees (○ = P15, ● = P30)

classes ($r = 0.99$, $P < 0.001$), based on the means of the six combinations of treatments (prox. × L, int. × M, dist. × S) in Table 1. There is no evidence either, from the correlation analysis, of any differences between plants in respect of these relationships. The regression line in Fig. 3 ($P < 0.001$) emphasises the strength of the coincidence of the distributions.

The slight excess of the small fragments in P15 is thought to be partly due to inaccuracies in the measurements of the small fragments and partly also to the fact that in P15 the range of fragment sizes only extended over the range 0.3–2.2 μ, whereas the groupings of L, M and S was made on the same basis as for P30, where the range extended from 0.3–2.7 μ. This method of assignment to the size groups may have introduced some bias in favour of the small and medium P15 fragment classes, at the expense of the large.

Chromosome breakage

The U-type exchanges to which we have referred above represent but one of several different kinds of error

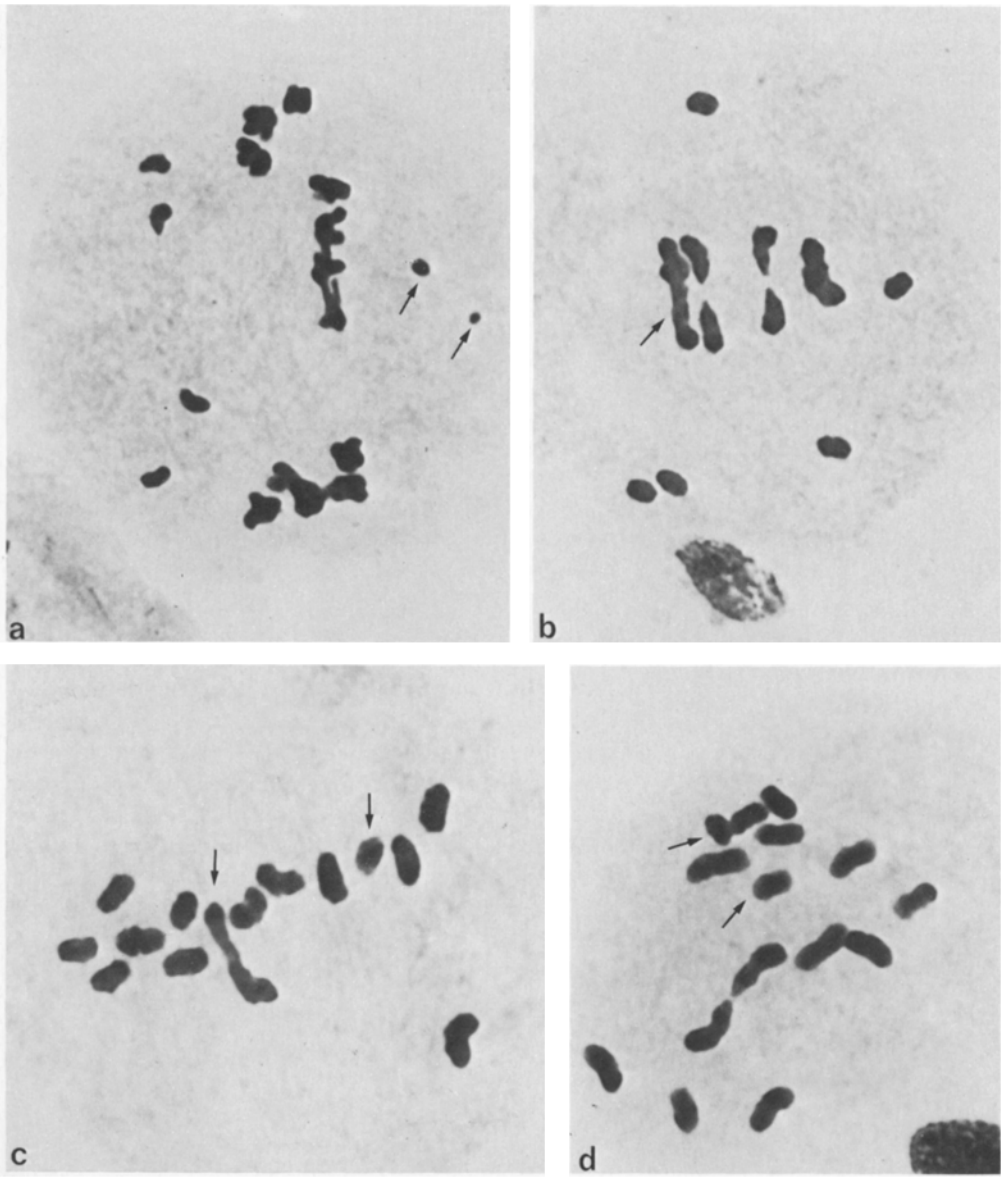

Fig. 4 a – d. Meiosis in two plants showing various forms of chromosome breakage. **a** AI in P34/1/30 showing a complex configuration involving three chromosomes held together by two bridges: the fragments deriving from the U-type exchanges are arrowed. **b** MI in P21/8 showing a linear interchange trivalent (*arrowed*). **c** MI in P21/8 with twelve univalents and an unequal rod bivalent and fragment (*both arrowed*): this configuration results from a chromosome break in one of the homologues in the arm not involved in chiasma formation. **d** MI in P21/8 with a single rod bivalent and twelve univalent chromosomes, one of which has broken into two parts (*arrowed*)

breakage events revealed among the early generation segregating families and the later generation established lines within the breeding scheme. In this section we make a brief mention of the occurrence and nature of some other kinds of chromosome breakages.

Multivalents. In five different inbred plants, one from the S1 and four from the S2, a single multivalent was observed in a small number of pmcs at MI (Fig. 4b). The frequencies, based on a sample of 100 MI cells were as follows: P21/8, 6; P15/1/1, 5; P34/1/6, 2; P34/1/26, 9; P34/1/30, 3. For several reasons the plants concerned were not considered to be interchange heterozygotes: (i) there is no ancestry of interchange within their pedigrees, (ii) the frequencies of multivalents were low and (iii) the form of the multivalents varied from one pmc to another within plants. The presence of multivalents can be explained either by the occurrence of spontaneous interchanges or by nonhomologous pairing. We have no direct evidence either way but, on

account of the ubiquitous occurrence of breakage in some form or other throughout the inbred pedigrees, it seems likely that the multivalents result from spontaneous interchange. Indeed two of the plants (P21/8 and P15/1/1) showed other types of breakage at MI, as described below, and in the remaining three B+Fs were also observed at AI.

In P34/1/30 an AI pmc was observed in which three chromosomes were involved in a B+F configuration: two chromatids of one chromosome have both been involved in a U-type exchange with one chromatid from each of two other chromosomes, to give two bridges and two fragments of differing sizes (Fig. 4a). Evidently two errors have occurred – a spontaneous interchange (or nonhomologous pairing) combined with two U-type exchanges.

Extensive chromosome breakage. P21/8 and P15/1/1 exhibited breakage errors of a more general kind. In P15/1/1 only a few pmcs were affected but in P21/8 breakage was observed in 42.3% of cells at MI, and 45.3% at AI. In the MI pmcs 12.8% had multiple breakages which were often difficult to interpret, and 29.6% showed chromosome-type breaks (Fig. 4c, d). Of this latter group 20% involved homologous pairs, either as a bivalent or an univalent (Fig. 4c and d respectively), and 9.6% involved non-homologues, either as interchanges (7.6%) or transpositions (2%). In 0.4% of MI cells chromatid-type breaks were seen. At AI chromosome and chromatid breakage was detected with equal frequency: the latter group included B+F configurations as well as chromatid fragments without bridges. In addition 5.7% of the AI cells showed side-arm bridges.

At AII breakage was observed in 21% of the cells. Of these 13.3% had chromatid bridges and in the rest one or more chromatid fragments could be seen. Ten percent of the AII cells contained micronuclei and this, together with data obtained from screening dyads and tetrads indicated that most of the products of breakage were excluded as micronuclei at the second meiotic division (68% of tetrads had micronuclei compared with 20% of dyads).

Although U-type reunion was involved in some of the breakage events seen in P21/8 it is clear that this plant was very different from the others described with U-type exchange events, which appeared to have errors associated with chiasma formation only, and that in P21/8 there is a much more fundamental and extensive breakage syndrome. On this account we might therefore expect the distribution of break-points along the chromosome to be different in P21/8. The distribution of the lengths of 101 fragments (from chromosome-type breaks) measured at MI are given in Fig. 5. A sample of univalent chromosomes were also measured and the

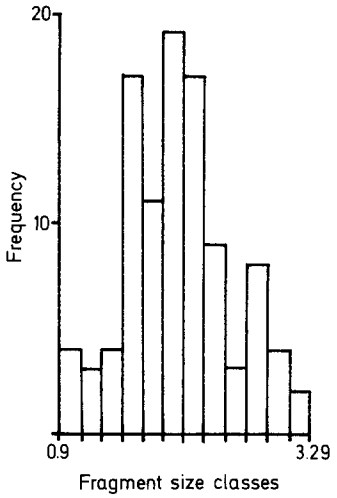

Fig. 5. Distribution of chromosome fragment lengths measured at MI in P21/8. The fragments are grouped into classes 0.19 μ in length

average length calculated as 3.53 μ, with a range of 2.80 to 4.30 μ. Comparing the mean and range of chromosome lengths with the fragment sizes given in Fig. 5 it can be seen that more than half of the fragments were about half the size of the chromosomes. Sixty percent of the fragments were between 1.50–2.49 μ and the mean length was 2.02 μ. Since the chromosomes are mostly metacentrics and submetacentrics it follows that most breakages occurred at, or near to, the centromere. This fits in with the observation that many of the fragments appeared to be telocentric or nearly so.

Discussion

The high incidence and widespread occurrence of B+Fs amongst the inbred lines of *L. perenne* can be taken as another manifestation of the breakdown of control over meiosis due to the accumulation of homozygous combinations of polygenes with deleterious effects. Such effects have also been reported in other material, in particular in rye (Rees and Thompson 1955; Giraldez and Lacadena 1978) and in the radish (Dayal 1979).

For the reasons which have been given, and which have previously been discussed in detail by other authors (Lewis and John 1965; Jones 1968), the B+Fs are interpreted as the result of U-type exchanges. Although a causal relationship between U-type exchange and chiasma formation has yet to be established there is evidence that the two phenomena share a common basis of occurrence and the most likely interpretation for the co-incidence of their distribution is that U-type exchanges arise as errors in chiasma

formation. Evidence supporting this hypothesis comes from the demonstration in rye of a correlation between the distribution of chiasmata, as seen at MI, and the distribution of U-type exchanges as estimated by measuring the size of the acentric fragments liberated at AI (Jones 1968, 1969). The present work, in perennial ryegrass, confirms this correlation through a study of the distribution of U-type in S5 pedigrees with contrasting patterns of chiasma distribution. The relationship also provides us with some evidence, albeit indirect, that the change in chiasma position observed over generations of inbreeding, and between the pedigrees (Karp and Jones 1983), represents a real shift in the sites of chiasma formation and is not simply the result of some artifact, or due to differences in degrees of terminalisation.

Giraldez and Lacadena (1978) have argued that in inbred rye there is a relationship between chiasma frequency, chiasma distribution and the frequency of chiasma errors (i.e. U-types) and they present evidence for significant correlations between these characters to show that as chiasma frequency declines both chiasma error frequency and distal localisation of chiasmata increase. They forward the suggestion that a single controlling system is involved and that the anomalies observed are secondary effects of the failure of some preconditions for exchange.

In *L. perenne* there can be no doubt either that the incidence of B+F formation must increase as inbreeding progresses, and in this respect there is likely to be an overall negative correlation with pmc chiasma frequency. Our data on B+Fs however are not as extensive as those for the other characters concerning chiasma frequency and distribution (parts 1 and 2), and we are unable to test out this relationship. What we can say however, from an assortment of early generation segregants and S5 plants that were analysed, is that while there may well be an overall negative correlation between the two variables, over generation of inbreeding, it certainly does not hold when more detailed comparisons are made. B+Fs were found in some plants with relatively high chiasma frequencies, and also at widely different levels in other plants with identical chiasma frequencies: in P34/1/6 and P34/1/23 (siblings of an S2 family) for example, the B+F frequencies at AI were 5.9% and 12.6% respectively, while the corresponding chiasma frequency means were virtually identical at 7.3 and 7.0. In other words, although the sites of formation of chiasmata and U-type exchanges coincide there is not necessarily a correlation of their frequencies; neither is there any particular relationship between the incidence of B+Fs and the pattern of chiasma distribution. Notwithstanding the absence of critical data on this point we would venture to suggest that the result is not altogether unexpected. There is no compelling reason to believe that homozygous gene combinations which find expression as U-type exchanges, and which segregate out during inbreeding, are the same ones, or that they should segregate out in the same pattern, as those determining chiasma frequency per se.

The cases presented on chromosome breakage can be added to a catalogue of those known in other plant species. Spontaneous breakage events in plants sampled from natural populations have been described, for example, in *Paeonia californica* (Walters 1956), *Podophyllum peltatum* (Newman 1967), *Claytonia virginica* (Star 1970), and *Najas marina* (Viinikka and Kotimäki 1977). In experimental material they have also been described in *Scilla siberica* (Rees 1952), inbred rye, *Secale cereale* (Rees and Thompson 1955; Rees 1962) and *Pisum sativum* (Klein and Baquar 1972), amongst others.

P21/8 is of interest on two accounts: firstly the level of meiotic breakage is much higher than is generally found in such phenotypes and secondly, the breakages are localised in the region of the centromere. There is no correspondence between the distribution of breakage with that of chiasmata, as was found in rye (Rees and Thompson 1956); the situation is more reminiscent of that found at pollen grain mitosis in *Tulipa sylvestris* where spontaneous breaks were found to occur close to the centromere (Darlington and Upcott 1941).

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